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Vaccination with ALVAC and AIDSVAX to Prevent HIV-1 Infection in Thailand

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ABSTRACT

BACKGROUND

The development of a safe and effective vaccine against the human immunodeficiency virus type 1 (HIV-1) is critical to pandemic control.

METHODS

In a community-based, randomized, multicenter, double-blind, placebo-controlled efficacy trial, we evaluated four priming injections of a recombinant canarypox vector vaccine (ALVAC-HIV [vCP1521]) plus two booster injections of a recombinant glycoprotein 120 subunit vaccine (AIDSVAX B/E). The vaccine and placebo injections were administered to 16,402 healthy men and women between the ages of 18 and 30 years in Rayong and Chon Buri provinces in Thailand. The volunteers, primarily at heterosexual risk for HIV infection, were monitored for the coprimary end points: HIV-1 infection and early HIV-1 viremia, at the end of the 6-month vaccination series and every 6 months thereafter for 3 years.

RESULTS

In the intention-to-treat analysis involving 16,402 subjects, there was a trend toward the prevention of HIV-1 infection among the vaccine recipients, with a vaccine efficacy of 26.4% (95% confidence interval [CI], −4.0 to 47.9; P=0.08). In the per-protocol analysis involving 12,542 subjects, the vaccine efficacy was 26.2% (95% CI, −13.3 to 51.9; P=0.16). In the modified intention-to-treat analysis involving 16,395 subjects (with the exclusion of 7 subjects who were found to have had HIV-1 infection at baseline), the vaccine efficacy was 31.2% (95% CI, 1.1 to 52.1; P=0.04). Vaccination did not affect the degree of viremia or the CD4+ T-cell count in subjects in whom HIV-1 infection was subsequently diagnosed.

CONCLUSIONS

This ALVAC-HIV and AIDSVAX B/E vaccine regimen may reduce the risk of HIV infection in a community-based population with largely heterosexual risk. Vaccination did not affect the viral load or CD4+ count in subjects with HIV infection. Although the results show only a modest benefit, they offer insight for future research. (ClinicalTrials.gov number, NCT00223080.)

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In the late 1980s in Thailand, there was a dramatic increase in the prevalence of infection with the human immunodeficiency virus type 1 (HIV-1) in sentinel surveillance cohorts.[1-3] Initially, these groups consisted of injection-drug users and commercial sex workers; they were subsequently expanded to include persons in the general population. By 1995, the overall seroprevalence of HIV-1 reached a peak of 3.7% among conscripts in the Royal Thai Army and of 12.5% among conscripts from Northern Thailand.[2,4,5] The Thai Ministry of Public Health responded with an effective HIV-prevention campaign, and the number of new HIV-1 infections per year decreased from an estimated 143,000 in 1990 to 14,000 in 2007.[2,4,6-9] The persistence of new infection despite these measures led public health officials to conclude that an HIV vaccine, within the context of a broader HIV-prevention program, was needed for better control of the epidemic.

A number of trials of various subtype B canarypox–HIV vector primes and boosters containing subunit glycoprotein 120 or 160 (gp120 or gp160) established the prime–boost concept as a candidate for advanced testing.[10-13] Canarypox-based prime–boost regimens induced both cellular and humoral responses, but CD8+ responses on enzyme-linked immunosorbent spot (ELISPOT) assay were low,[12] and the presence of primary isolate neutralizing antibody was not consistently detected.[14-18]

A series of phase 1 and 2 trials of HIV vaccines involving more than 1000 Thai volunteers was undertaken, with products matching the circulating HIV-1 subtypes B and CRF01_AE.[8,17-22] Although a phase 3 trial of VaxGen bivalent gp120 AIDSVAX B/E vaccine alone involving injection-drug users showed no effect on HIV-1 acquisition,[21] a phase 2 trial of an ALVAC-HIV (vCP1521) prime with an AIDSVAX B/E boost showed induction of prespecified cellular and humoral immune responses and was consistent with criteria for advancement to a large test-of-concept study.[17] In October 2003, our study was initiated in a population at community risk for HIV infection.[8]

METHODS

STUDY DESIGN AND POPULATION

This study was a community-based, randomized, multicenter, double-blind, placebo-controlled efficacy trial of the prime–boost combination vaccines containing ALVAC-HIV (vCP1521) (Sanofi Pasteur) and AIDSVAX B/E (Global Solutions for Infectious Diseases). For details regarding the vaccines and placebo, see the Supplementary Appendix, available with the full text of this article at NEJM.org. The study was designed to evaluate two coprimary end points: the prevention of HIV-1 infection and the effect of vaccination on the early viral load after infection. The trial was conducted through facilities of the Thai Ministry of Public Health in Rayong and Chon Buri provinces. From September 2003 through December 2005, a total of 16,402 volunteers were enrolled.

Thai men and women who were between the ages of 18 and 30 years and who were not infected with HIV were recruited from the community without regard to HIV risk (i.e., community risk). Written informed consent was obtained from all volunteers, who were required to pass a written test of understanding. Women were counseled to practice effective contraception until 3 months after the last vaccination; pregnant and breast-feeding women were excluded.

STUDY OVERSIGHT

The protocol was reviewed by the ethics committees of the Ministry of Public Health, the Royal Thai Army, Mahidol University, and the Human Subjects Research Review Board of the U.S. Army Medical Research and Materiel Command. It was also independently reviewed and endorsed by the World Health Organization and the Joint United Nations Program on HIV/AIDS and by the AIDS Vaccine Research Working Group of the National Institute of Allergy and Infectious Diseases at the National Institutes of Health. The manufacturers were full trial collaborators and were a part of the phase 3 trial steering committee.

STUDY PROCEDURES

The study vaccines were administered at baseline (day 0), 4 weeks (prespecified range, 3 to 7), 12 weeks (range, 10 to 15), and 24 weeks (range, 21 to 28). The ALVAC-HIV (vCP1521) vaccine was administered at each of the four visits. Boosting with AIDSVAX B/E occurred at weeks 12 and 24. For 3 days after each dose of vaccine, subjects reported local and systemic vaccine reactions on a diary card. All other adverse and serious adverse events were documented at each visit and were graded on a scale that is used for rating adverse events associated with vaccines, as recommended by the Division of Acquired Immunodeficiency

Syndrome of the National Institute of Allergy and Infectious Diseases (http://rcc.tech-res.com/safetyandpharmacovigilance). All subjects who underwent randomization were included in the safety analysis.

Women underwent urine testing for pregnancy throughout the vaccination phase. Pregnant volunteers did not receive further vaccinations. All volunteers were followed with the use of HIV testing at day 0, at 24 and 26 weeks, and every 6 months during the 3-year follow-up phase. Peripheral-blood mononuclear cells were isolated and archived in liquid nitrogen at 0, 6, 12, and 42 months. Assessment of behavior associated with an increased risk of HIV infection occurred at baseline, at week 26, and at each 6-month follow-up visit. HIV-prevention counseling was provided during each vaccination and post-test counseling visit.

PRIMARY END POINTS

We established the presence of HIV infection on the basis of repeated positive results on enzyme immunoassay and Western blotting, with two confirmatory HIV nucleic acid tests: the Amplicor HIV Monitor (version 1.5) assay (Roche) in Thailand and the Procleix HIV discriminatory assay (Novartis) in the United States. We performed three measurements of HIV-1 RNA within 6 weeks after serodiagnosis to determine the mean postinfection viral load. Infection time was defined as the midpoint between the last negative result and the first positive result of testing. An independent endpoints monitoring committee whose members were unaware of study-group assignments verified the accuracy of all diagnoses.

ASSESSMENT OF RISK

We assessed subjects' risk of HIV infection using a self-administered behavioral questionnaire at baseline and every 6 months thereafter. First, volunteers categorized themselves as being at high, moderate, or low risk for HIV infection. A second approach categorized subjects as being at high risk if they reported being at high risk or reported any high-risk behavior (e.g., needle sharing, multiple sex partners, commercial sex work, and symptoms of sexually transmitted disease). Volunteers were considered to be at low risk if they perceived their risk as low; if they reported that in the previous 6 months they had had no more than one sex partner and no sexual contact with a commercial sex worker, a partner of the same sex, an HIV-infected partner, a partner who used injection drugs, or a partner who had multiple partners; and if they reported having had no symptoms of a sexually transmitted disease or incarceration within 6 months before study entry. Moderate-risk subjects were considered to be at neither low nor high risk.

IMMUNOGENICITY ANALYSES

We analyzed plasma and cells from volunteers who did not have HIV infection at various time points after vaccination to evaluate immunogenicity. After removal of a small subgroup of samples for future matched case–control studies, we identified random samples and provided them in a blinded fashion to the Armed Forces Research Institute of Medical Sciences laboratory at a ratio of samples from the vaccine group to samples from the placebo group of approximately 4:1. The immunogenicity of the vaccine regimen was measured with the use of the following validated assays: interferon-γ ELISPOT and CD4+ and CD8+ intracellular cytokine staining for interferon-γ and interleukin-2 to Gag and Env; binding antibody to gp120 in the MN strain, gp120 in the A244 strain (CM244), and p24 Gag; and lymphoproliferation to gp120 MN, gp120 A244, and p24 (for details, see the Supplementary Appendix).[17,18,22-25]

STATISTICAL ANALYSIS

According to the study protocol, we conducted both intention-to-treat and per-protocol analyses. The intention-to-treat analysis included all subjects who underwent randomization. Because of the time between screening and vaccination and the possibility of acquiring HIV-1 infection during this interval, the protocol specified look-back testing of baseline plasma for HIV-1 RNA if the sample that was collected on the day of the fourth vaccination was HIV-seropositive. Seven persons who were enrolled and vaccinated were found to be positive for HIV-1 RNA at baseline. The per-protocol analysis included a subgroup of subjects in the intention-to-treat analysis who received the entire series of vaccinations within the defined time period, who remained eligible to participate in the study, and who did not have HIV infection at the time of the fourth vaccination. A separate subgroup analysis, called the modified intention-to-treat analysis, excluded the seven volunteers who were found to have HIV infection at baseline. This was used as the primary analysis

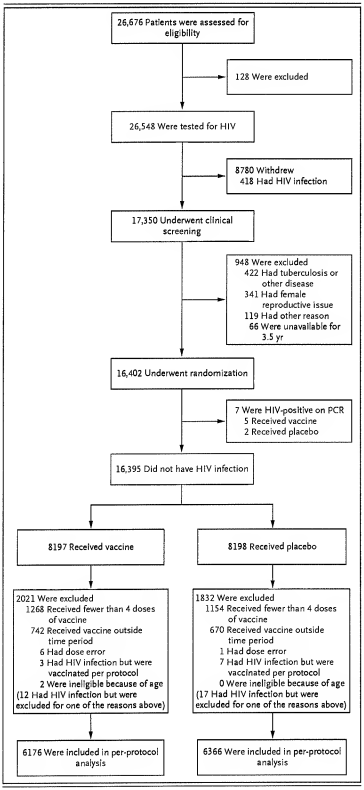

Figure 1. Enrollment and Outcomes.

During the course of the study, there were 15 HIV-1 infections in the vaccine group and 24 in the placebo group that were excluded from the final analysis. This left 12,542 volunteers (6176 in the vaccine group and 6366 in the placebo group) who received all four doses of vaccine within the prespecified time period, who were not excluded for the other reasons, and who did not have HIV-1 infection at visit 7 (per-protocol population).

at the time of the interim and final analyses and was prespecified in the final data-analysis plan that was approved 5 months before the unblinding of the study. (For details regarding the sample size calculation, randomization procedures, and calculation of vaccine efficacy, see the Supplementary Appendix.)

After the initiation of the trial, the effect of vaccination on early viral load was included as a coprimary end point, and the mean postinfection viral load was compared between vaccine and placebo recipients at the 1% level with the Wilcoxon statistic. The effect of selection bias was considered.[26]

The trial was monitored by an independent, international data and safety monitoring board, which met every 6 to 12 months (eight times during the trial) and reviewed the trial for safety and futility. At the interim analysis, the trial was reviewed for efficacy, safety, and futility. Statistical futility for the acquisition end point was examined with a trigger for early termination if the conditional power was less than 10%. All reported P values are two-tailed and have not been adjusted for multiple testing. A P value of less than 0.05 was considered to indicate statistical significance.

RESULTS

STUDY POPULATION

A total of 26,676 volunteers were screened and 16,402 were enrolled (intention-to-treat group) (Fig. 1). The 12,542 subjects who completed all vaccination visits on schedule and were not found to have HIV-1 infection after receiving the full vaccination regimen were included in the per-protocol analysis. Seven volunteers who were found to

Table 1. Baseline Characteristics of the Subjects (Modified Intention-to-Treat Population).

Variable	Vaccine (N=8197)	Placebo (N=8198)	All Subjects (N=16,395)
		number (percent)	
Sex			
Male	5033 (61.4)	5031 (61.4)	10,064 (61.4)
Female	3164 (38.6)	3167 (38.6)	6,331 (38.6)
Age group			
≤20 yr	2297 (28.0)	2246 (27.4)	4,543 (27.7)
21–25 yr	3633 (44.3)	3708 (45.2)	7,341 (44.8)
≥26 yr	2267 (27.7)	2244 (27.4)	4,511 (27.5)
Province			
Chon Buri	4107 (50.1)	4107 (50.1)	8,214 (50.1)
Rayong	4090 (49.9)	4091 (49.9)	8,181 (49.9)
Marital status			
Single	3353 (40.9)	3338 (40.7)	6,691 (40.8)
Married	4110 (50.1)	4169 (50.9)	8,279 (50.5)
Divorced	602 (7.3)	541 (6.6)	1,143 (7.0)
Widowed	50 (0.6)	64 (0.8)	114 (0.7)
Separated	82 (1.0)	86 (1.0)	168 (1.0)
No. of sex partners			
0	1864 (22.7)	1801 (22.0)	3,665 (22.4)
1	5428 (66.2)	5495 (67.0)	10,923 (66.6)
>1	619 (7.6)	620 (7.6)	1,239 (7.6)
Did not answer	280 (3.4)	273 (3.3)	553 (3.4)
Missing data	6 (0.1)	9 (0.1)	15 (0.1)
Risk group			
Low	3865 (47.2)	3924 (47.9)	7,789 (47.5)
Medium	2369 (28.9)	2292 (28.0)	4,661 (28.4)
High	1963 (23.9)	1982 (24.2)	3,945 (24.1)
Behavioral risk			
Needle sharing	68 (0.8)	65 (0.8)	133 (0.8)
No condom use			
With casual partner	497 (6.1)	439 (5.4)	936 (5.7)
With commercial sex worker	33 (0.4)	29 (0.4)	62 (0.4)
With same-sex partner	79 (1.0)	90 (1.1)	169 (1.0)
With HIV-infected partner	16 (0.2)	13 (0.2)	29 (0.2)
With partner who injects drugs	12 (0.1)	6 (0.1)	18 (0.1)
With multiple sex partners	128 (1.6)	130 (1.6)	258 (1.6)
Condom use with HIV-infected partner	113 (1.4)	114 (1.4)	227 (1.4)
Symptoms of an STD within past 6 mo*	246 (3.0)	233 (2.8)	479 (2.9)
Drug injection in jail	23 (0.3)	15 (0.2)	38 (0.2)
Occupation as a commercial sex worker	42 (0.5)	44 (0.5)	86 (0.5)
Occupation in the entertainment business	233 (2.8)	237 (2.9)	470 (2.9)

* STD denotes sexually transmitted disease.

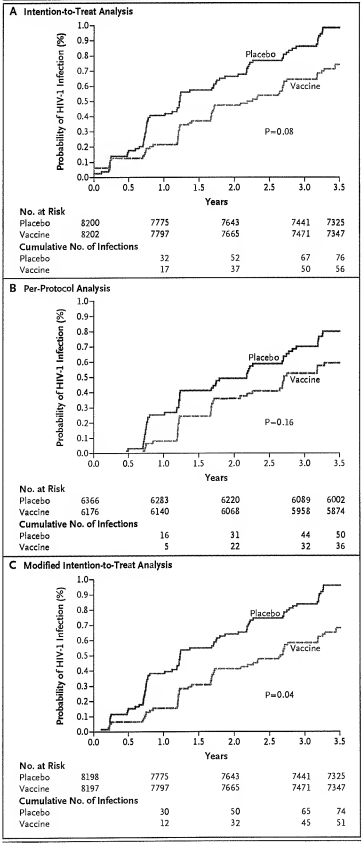

Figure 2. Kaplan–Meier Cumulative Rates of Infection, According to Type of Analysis.

The vaccination regimen was completed approximately 6 months after the first dose was administered. In the intention-to-treat analysis involving 16,402 subjects, the vaccine efficacy was 26.4% (95% confidence interval [CI], −4.0 to 47.9; P=0.08) (Panel A). In the per-protocol analysis involving 12,542 subjects, the vaccine efficacy was 26.2% (95% CI, −13.3 to 51.9; P=0.16) (Panel B). In the modified intention-to-treat analysis involving 16,395 subjects (excluding 7 subjects who were found to have had HIV infection at baseline), the vaccine efficacy was 31.2% (95% CI, 1.1 to 51.2; P=0.04) (Panel C).

be seropositive for HIV-1 on the first test after vaccination were determined by RNA testing to have been infected at enrollment and were not included in the modified intention-to-treat analysis, leaving 16,395 volunteers: 8197 in the vaccine group and 8198 in the placebo group. This group consisted of 10,064 men (61.4% of the subjects) and 6331 women (38.6%). Baseline characteristics were similar for selected variables, and there was no imbalance between the two groups in self-described risk behavior (Table 1).

There were no substantive changes in serial self-reports of risk behavior during the trial. No data were collected on the status of male circumcision or on serologic analyses for adenovirus type 5 or herpes simplex virus type 2.

There were 52,985 person-years of follow-up (15% more than planned). At 42 months, 14,672 of the volunteers (89.5%) had completed the trial and were HIV-seronegative.

ADVERSE EVENTS

Most local and systemic reactions to the vaccine were mild to moderate and reflected the findings of studies on the safety of these products that have been reported previously[12,17,27-29] (Fig. 1 in the Supplementary Appendix). Most reactions were mild to moderate and resolved within 3 days after vaccination. At least one adverse event was reported in 69.4% of subjects in the two study groups. The number of deaths and the frequency and severity of adverse events and serious adverse events were similar in the two groups (Table 1 in the Supplementary Appendix).

PRIMARY END POINTS

HIV-1 Infection

HIV-1 infection was diagnosed in 132 subjects (56 in the vaccine group and 76 in the placebo

Table 2. Rate of HIV Infection and Vaccine Efficacy, According to Selected Baseline Variables (Modified Intention-to-Treat Population).

Variable	Vaccine (N=8197)				Placebo (N=8198)				Vaccine Efficacy
	No. Evaluated	No. with Infection	No. of Person-Years	Rate	No. Evaluated	No. with Infection	No. of Person-Years	Rate	
				no./person-yr				*no./person-yr*	*% (95% CI)*
All subjects	7960	51	26,507	0.192	7988	74	26,478	0.279	31.2 (1.7 to 51.8)
Sex									
Male	4875	32	16,221	0.197	4885	43	16,179	0.266	25.8 (–17.3 to 53.0)
Female	3085	19	10,286	0.185	3103	31	10,300	0.301	38.6 (–8.6 to 65.3)
Age group									
≤20 yr	2228	12	7,358	0.163	2185	11	7,216	0.152	7.1 (–143.0 to 52.7)
21–25 yr	3517	20	11,713	0.171	3610	40	11,946	0.335	49 (12.8 to 70.2)
≥26 yr	2215	19	7,437	0.255	2193	23	7,316	0.314	18.7 (–49.3 to 55.7)
Living with partner									
Yes	4017	19	13,466	0.141	4083	34	13,612	0.25	43.5 (1.0 to 67.8)
No	3943	32	13,041	0.245	3905	40	12,866	0.311	21 (–25.7 to 50.4)
Risk group									
Low	3767	17	12,565	0.135	3837	29	12,798	0.227	40.4 (–8.5 to 67.2)
Medium	2297	12	7,642	0.157	2222	22	7,353	0.299	47.6 (–6.0 to 74.0)
High	1896	22	6,300	0.349	1929	23	6,327	0.364	3.7 (–72.7 to 46.3)

group) during 52,985 person-years of follow-up in the intention-to-treat analysis, in 86 subjects (36 in the vaccine group and 50 in the placebo group) during 36,720 person-years of follow-up in the per-protocol analysis, and in 125 subjects (51 in the vaccine group and 74 in the placebo group) during 52,985 person-years of follow-up in the modified intention-to-treat analysis. One subject in the placebo group who was identified by hospital record as being seropositive for HIV after dying from *Pneumocystis jirovecii* pneumonia was included in the analysis before the unblinding of the study. This diagnosis of HIV-1 infection was the only one that occurred outside planned procedures.

With the use of the Cox proportional-hazards method, the observed vaccine efficacy was 26.4% (95% confidence interval [CI], –4.0 to 47.9; P=0.08) in the intention-to-treat analysis (Fig. 2A); 26.2% (95% CI, –13.3 to 51.9; P=0.16) in the per-protocol analysis (Fig. 2B); and 31.2% (95% CI, 1.1 to 52.1; P=0.04 by the O'Brien–Fleming method) in the modified intention-to-treat analysis (Fig. 2C). Because HIV testing was done at week 24, it is not possible to discern which dose of vaccine might have been associated with an early effect. The overall observed effect in the modified intention-to-treat analysis was evaluated with the use of several different analyses: event rates by Barnard's test (P=0.04), the log-rank test (P=0.04), the Wilcoxon test (P=0.03), modification of the time-to-seroconversion end point (P=0.04), exclusion of the in-hospital diagnosed case (P=0.05), and analysis of interval-censored data (P=0.04).

Covariates were analyzed for the populations with similar results. Simultaneous adjustment for sex, age, living with a partner, and baseline risk factors did not affect estimates of vaccine efficacy, even though between-group differences in age, living with a partner, and baseline risk factors were significant. Subgroup analyses revealed no significant heterogeneity in vaccine efficacy according to baseline variables (Table 2).

There were 86 HIV-1 infections in the per-protocol population and 125 infections in the modified intention-to-treat population. There were three categories into which the 39 subjects with HIV-1 infection who were excluded from the per-protocol population could be organized: 10 subjects (3 in the vaccine group and 7 in the placebo group) were infected during the vaccination phase and received all vaccinations on schedule; 10 subjects (3 in the vaccine group and 7 in

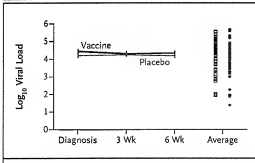

Figure 3. Viral Loads in Subjects with Early HIV-1 Infection.

The receipt of vaccine did not have a significant effect on the viral load in subjects who were found to have early HIV-1 infection. On the left are the mean $\log_{10}$ viral loads at three visits during a 6-week period for subjects who were included in the intention-to-treat analysis. The data points at the right show the distribution of viral loads in the vaccine group (mean, 4.36 $\log_{10}$ copies per milliliter) and the placebo group (mean, 4.21 $\log_{10}$ copies per milliliter) ($P=0.09$). There was no significant between-group difference in viral load in either the per-protocol analysis ($P=0.47$) or the modified intention-to-treat analysis ($P=0.24$).

the placebo group) were infected after the vaccination phase and received all vaccinations, but one or more vaccinations were not administered during the prespecified window; and 19 subjects (9 in the vaccine group and 10 in the placebo group) were infected after the vaccination phase but did not receive all vaccinations.

Postinfection Viral Load and CD4+ T-Cell Count

There was no significant difference in the mean viral load among subjects who were found to have HIV infection in the vaccine group, as compared with those in the placebo group. The mean viral-load values were 4.36 $\log_{10}$ copies per milliliter in the vaccine group and 4.21 $\log_{10}$ copies per milliliter in the placebo group ($P=0.09$ by the Wilcoxon test) in the intention-to-treat analysis (Fig. 3). The viral-load values were 4.24 $\log_{10}$ copies per milliliter in the vaccine group and 4.19 $\log_{10}$ copies per milliliter in the placebo group in the per-protocol analysis ($P=0.47$) and 4.30 $\log_{10}$ copies per milliliter and 4.20 $\log_{10}$ copies per milliliter, respectively, in the modified intention-to-treat analysis ($P=0.24$).

In all three analyses, there were no significant between-group differences in postinfection CD4+ T-cell counts. The mean early postinfection CD4+ T-cell count was 541 cells per microliter in the vaccine group and 568 cells per microliter in the placebo group in the intention-to-treat analysis ($P=0.47$ by the Wilcoxon test), 572 cells per microliter in the vaccine group and 532 cells per microliter in the placebo group in the per-protocol analysis ($P=0.72$), and 555 cells per microliter in the vaccine group and 568 cells per microliter in the placebo group in the modified intention-to-treat analysis ($P=0.76$).

IMMUNOGENICITY

Vaccination induced an HIV-specific response, as measured by the production of interferon-γ by T cells when exposed to either Env or Gag antigen on ELISPOT assay, in 19.7% of volunteers 6 months after the final dose of vaccine was administered (Table 3 and the Supplementary Appendix). This result was similar to the rate of 17% in the phase 2 trial (de Souza MS: personal communication). Response rates for CD4+ Env-specific intracellular cytokine staining were higher in the vaccine group than in the placebo group. Rates of positivity in the gp120 and p24 binding-antibody assays and the lymphoproliferation assay were similar to those in the phase 2 study.[17] Binding antibody for Env was nearly uniformly present, with the reciprocal of the geometric mean titer (GMT^{-1}) of 31,207 for the MN strain and 14,558 for the A244 strain, whereas p24 responses were less frequent (GMT^{-1}, 138) (for details, see the Supplementary Appendix). The median lymphocyte stimulation index (LSI) was 2 for all subjects at baseline and subsequently in placebo recipients. The LSI was significantly higher in vaccine recipients (median LSI, 24 for gp120 MN, 32 for A244, and 4 for p24).

DISCUSSION

In this clinical trial, we evaluated the efficacy of ALVAC-HIV priming and AIDSVAX B/E boosting for the prevention of HIV-1 infection in more than 16,000 young Thai adults at community risk for such infection. In the intention-to-treat group (which included seven subjects who were found to have had HIV-1 infection at baseline), there was a trend toward prevention of infection with the vaccine regimen. In the per-protocol analysis, which excluded 30% of the end points and person-years of follow-up, the results were not significant. However, after the exclusion of the subjects who were infected with HIV-1 before vaccination, the modified intention-to-treat analysis showed a significant, though modest, reduc-

Table 3. Immunogenicity Analyses at Baseline and 12 Months.*

Assay and Antigen	Baseline	12 Months	
		Vaccine	Placebo
	no. positive/total no. (%)	*no. positive/total no. (%)*	*no. positive/total no. (%)*
ELISPOT			
Gag	7/194 (3.6)	13/156 (8.3)	3/41 (7.3)
Env	7/198 (3.5)	25/157 (15.9)	3/41 (7.3)
Gag or Env	8/198 (4.0)	31/157 (19.7)	3/41 (7.3)
Intracellular cytokine staining			
CD8 Gag	11/200 (5.5)	11/144 (7.6)	4/56 (7.1)
CD8 Env	15/200 (7.5)	16/144 (11.1)	8/56 (14.3)
CD4 Gag	0/200	2/144 (1.4)	0/56
CD4 Env	4/200 (2.0)	49/144 (34.0)†	2/56 (3.6)
Binding antibody‡			
gp120 MN	8/200 (4.0)	140/142 (98.6)†	0/58
gp120 A244	1/200 (0.5)	140/142 (98.6)†	0/58
p24	2/200 (1.0)	74/142 (52.1)†	0/58
Lymphoproliferation‡§			
gp120 MN	23/96 (24.0)	62/71 (87.3)†	5/25 (20.0)
gp120 A244	12/96 (12.5)	64/71 (90.1)†	4/25 (16.0)
p24	19/96 (19.8)	35/71 (49.3)¶	4/25 (16.0)

* All analyses were performed on samples collected at baseline (visit 1) and at 12 months (visit 9), unless otherwise specified.
† P<0.001 for the between-group comparison.
‡ These analyses were performed at 6.5 months (visit 8), 2 weeks after the administration of the fourth dose of vaccine.
§ Lymphoproliferation was measured with the use of the lymphocyte stimulation index (LSI). Values are for subjects who had an LSI of 5 or more.
¶ P=0.001 for the between-group comparison.

tion in the rate of HIV-1 infection, as compared with placebo.

Taken together, these data are consistent with a modest protective effect of vaccine in this study. However, there was no significant difference in the HIV-1 viral load or the postinfection CD4+ count between the two study groups. A simple, combined analysis of phase 1 and 2 ALVAC-HIV and gp120 prime–boost studies showed a rate of HIV-1 infection of 0.59 per 100 person-years in the vaccine group and 1.2 per 100 person-years in the placebo group, for a vaccine efficacy of 50% (95% CI, −39 to 80), a difference that was not significant; the results also showed no effect on viral load.[30] In nonhuman primates, ALVAC-SIV appeared to protect neonatal macaques against infection from milk containing a low dose of simian immunodeficiency virus (SIV).[31] However, ALVAC-SIV did not prevent infection from a more intense challenge exposure, although it did reduce the viral load and delay disease progression.[32,33]

Our trial did not have sufficient power to determine whether there was an effect of risk stratification on either disease acquisition or vaccine efficacy, and none of the observed heterogeneity achieved significance. Previous efficacy trials of HIV vaccines in higher-risk populations have not shown an effect on disease acquisition. Bivalent subtype B AIDSVAX B/B gp120 did not protect high-risk men who have sex with men,[34-36] and AIDSVAX B/E did not protect Thai injection-drug users[21] from infection with HIV-1. The Step trial of Merck recombinant adenovirus type 5 (rAd5) HIV-1 vaccine containing subtype B *gag*, *pol*, and *nef* in high-risk men who have sex with men was stopped because of futility and possibly higher rates of infection in vaccine recipients.[37]

An immunologic correlate with protection from HIV-1 infection has not been determined at this time. Though early studies of canarypox–gp120 subunit prime–boost regimens were promising,[10-13] advanced-phase testing of subtype B ALVAC-HIV (vCP1452) and AIDSVAX B/B was can-

celed because CD8+ reactivity on ELISPOT was too low.[12] The vaccines that were used in our trial showed a level of immunogenicity that was similar to levels reported previously.[17] Additional studies with the use of more recently developed immunogenicity assays are planned in order to determine their suitability for correlates analyses.[38-41] Further insight may be gained through molecular-sieve analysis of breakthrough infections with the use of single-genome amplification.[42]

Although our study provided preliminary evidence that an HIV vaccine regimen has the potential to prevent infection, it did not have the power to address two intriguing considerations: vaccine efficacy may have decreased over the first year after vaccination, and vaccine efficacy may have been greater in persons at lower risk for infection (Fig. 2 and Table 2). These issues deserve greater attention in future studies. We do not understand the immune mechanisms mediating the results that we observed. The ALVAC-HIV and AIDSVAX B/E prime–boost regimen induces a broad constellation of immune responses against HIV-1, including T-cell–line adapted neutralizing antibody (71% with response), antibody-directed, cell-mediated cytotoxicity, CD4+ lymphoproliferation (61% with response to gp20 MN, 63% with response to gp120 CM244), and CD8+ T cells (24% with response to ^{51}Cr-release cytotoxic T-cell assay; 17% with positive response on ELISPOT),[17,33,43] but these may not be the relevant responses. Understanding the potential immunologic correlates of protection will be a principal research focus. The data also do not answer the related question of whether it was a single vaccine or the combination of vaccines that induced a potentially protective immune response. Previous studies have suggested that prime–boost combinations induce qualitative or quantitative protective immune responses that are not seen with either vaccine alone, but the current data do not address this question.[28,44]

Finally, our study supports the possibility that immunologic mechanisms mediating protection against HIV may be different from those mediating early postinfection control of viral replication.[45,46] Taken together, these considerations underscore the opportunities afforded by the efficacy testing of HIV vaccines in human subjects in providing an objective context for review of existing methods of vaccine design, immunogenicity testing, and animal models.

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Drs. Gurunathan, Tartaglia, and McNeil report being employees of Sanofi Pasteur, and Dr. Tartaglia reports having an equity interest in the company. No other potential conflict of interest relevant to this article was reported.

The opinions expressed in this article are those of the authors and do not represent the official views of the Department of Health and Human Services, the National Institute of Allergy and Infectious Diseases, the Centers for Disease Control and Prevention, the Department of Defense, or Department of the Army.

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APPENDIX

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REFERENCES

1. Brown T, Peerapatanapokin W. The Asian epidemic model: a process model for exploring HIV policy and programme alternatives in Asia. Sex Transm Infect 2004;80:Suppl 1:i19-i24.
2. Phoolcharoen W. HIV/AIDS prevention in Thailand: success and challenges. Science 1998;280:1873-4.
3. Weniger BG, Limpakarnjanarat K, Ungchusak K, et al. The epidemiology of HIV infection and AIDS in Thailand. AIDS 1991;5 Suppl 2:S71-S85. [Erratum, AIDS 1993;7:following 147.]
4. Nelson KE, Celentano DD, Eiumtrakol S, et al. Changes in sexual behavior

and a decline in HIV infection among young men in Thailand. N Engl J Med 1996;335:297-303.
5. Nitayaphan S, Brown AE. Preventive HIV vaccine development in Thailand. AIDS 1998;Suppl:S155-S161.
6. Hanenberg RS, Rojanapithayakorn W, Kunasol P, Sokal DC. Impact of Thailand's HIV-control programme as indicated by the decline of sexually transmitted diseases. Lancet 1994;344:243-5.
7. Nelson KE, Celentano DD, Suprasert S, et al. Risk factors for HIV infection among young adult men in northern Thailand. JAMA 1993;270:955-60.
8. Rerks-Ngarm S, Brown AE, Khamboonruang C, Thongcharoen P, Kunasol P. HIVAIDS preventive vaccine 'prime-boost' phase III trial: foundations and initial lessons learned from Thailand. AIDS 2006;20:1471-9.
9. National AIDS Prevention and Alleviation Committee. UNGASS country progress report: Thailand. Reporting Period January 2006 - December 2007. (Accessed October 19, 2009, at http://data.unaids.org/pub/Report/2008/thailand_2008_country_progress_report_en.pdf.)
10. Belshe RB, Gorse GJ, Mulligan MJ, et al. Induction of immune responses to HIV-1 by canarypox virus (ALVAC) HIV-1 and gp120 SF-2 recombinant vaccines in uninfected volunteers. AIDS 1998;12:2407-15.
11. Belshe RB, Graham BS, Keefer MC, et al. Neutralizing antibodies to HIV-1 in seronegative volunteers immunized with recombinant gp120 from the MN strain of HIV-1. JAMA 1994;272:475-80.
12. Russell ND, Graham BS, Keefer MC, et al. Phase 2 study of an HIV-1 canarypox vaccine (vCP1452) alone and in combination with rgp120: negative results fail to trigger a phase 3 correlates trial. J Acquir Immune Defic Syndr 2007;44:203-12.
13. The AIDS Vaccine Evaluation Group 022 Protocol Team. Cellular and humoral immune responses to a canarypox vaccine containing human immunodeficiency virus type 1 env, gag, and pro in combination with rgp120. J Infect Dis 2001;183:563-70.
14. Mascola JR, Snyder SW, Weislow OS, et al. Immunization with envelope subunit vaccine products elicits neutralizing antibodies against laboratory-adapted but not primary isolates of human immunodeficiency virus type 1. J Infect Dis 1996;173:340-8.
15. Verrier F, Burda S, Belshe R, et al. A human immunodeficiency virus prime-boost immunization regimen in humans induces antibodies that show intercalde cross-reactivity and neutralize several X4-, R5-, and dualtropic clade B and C primary isolates. J Virol 2000;74:10025-33.
16. Zolla-Pazner S, Xu S, Burda S, Duliege AM, Excler JL, Clements-Mann ML. Neutralization of syncytium-inducing primary isolates by sera from human immunodeficiency virus (HIV)-uninfected recipients of candidate HIV vaccines. J Infect Dis 1998;178:1502-6.
17. Nitayaphan S, Pitisuttithum P, Karnasuta C, et al. Safety and immunogenicity of an HIV subtype B and E prime-boost vaccine combination in HIV-negative Thai adults. J Infect Dis 2004;190:702-6.
18. Thongcharoen P, Suriyanon V, Paris RM, et al. A phase 1/2 comparative vaccine trial of the safety and immunogenicity of a CRF01_AE (subtype E) candidate vaccine: ALVAC-HIV (vCP1521) prime with oligomeric gp160 (92TH023/LAI-DID) or bivalent gp120 (CM235/SF2) boost. J Acquir Immune Defic Syndr 2007;46:48-55.
19. Nitayaphan S, Khamboonruang C, Sirisophana N, et al. A phase I/II trial of HIV SF2.gp120/MF59 vaccine in seronegative Thais. Vaccine 2000;18:1448-55.
20. Pitisuttithum P, Berman PW, Phonrat B, et al. Phase I/II study of a candidate vaccine designed against the B and E subtypes of HIV-1. J Acquir Immune Defic Syndr 2004;37:1160-5. [Erratum, J Acquir Immune Defic Syndr 2004;37:1669.]
21. Pitisuttithum P, Gilbert P, Gurwith M, et al. Randomized, double-blind, placebo-controlled efficacy trial of a bivalent recombinant glycoprotein 120 HIV-1 vaccine among injection drug users in Bangkok, Thailand. J Infect Dis 2006;194:1661-71.
22. Pitisuttithum P, Nitayaphan S, Thongcharoen P, et al. Safety and immunogenicity of combinations of recombinant subtype E and B human immunodeficiency virus type 1 envelope glycoprotein 120 vaccines in healthy Thai adults. J Infect Dis 2003;188:219-27.
23. Kibuuka H, Kimutai R, Maboko L, et al. A phase I/II study of a multiclade HIV-1 DNA plasmid prime and recombinant Adenovirus-type 5 boost vaccine in HIV uninfected East Africans. J Infect Dis (in press).
24. Currier JR, Kuta EG, Turk E, et al. A panel of MHC class I restricted viral peptides for use as a quality control for vaccine trial ELISPOT assays. J Immunol Methods 2002;260:157-72.
25. Fu TM, Dubey SA, Mehrotra DV, et al. Evaluation of cellular immune responses in subjects chronically infected with HIV type 1. AIDS Res Hum Retroviruses 2007;23:67-76.
26. Gilbert PB, Bosch RJ, Hudgens MG. Sensitivity analysis for the assessment of causal vaccine effects on viral load in HIV vaccine trials. Biometrics 2003;59:531-41.
27. Belshe RB, Stevens C, Gorse GJ, et al. Safety and immunogenicity of a canarypox-vectored human immunodeficiency virus Type 1 vaccine with or without gp120: a phase 2 study in higher- and lower-risk volunteers. J Infect Dis 2001;183:1343-52.
28. Clements-Mann ML, Weinhold K, Matthews TJ, et al. Immune responses to human immunodeficiency virus (HIV) type 1 induced by canarypox expressing HIV-1MN gp120, HIV-1SF2 recombinant gp120, or both vaccines in seronegative adults. NIAID AIDS Vaccine Evaluation Group. J Infect Dis 1998;177:1230-46.
29. Gupta K, Hudgens M, Corey L, et al. Safety and immunogenicity of a high-titered canarypox vaccine in combination with rgp120 in a diverse population of HIV-1-uninfected adults: AIDS Vaccine Evaluation Group Protocol 022A. J Acquir Immune Defic Syndr 2002;29:254-61.
30. Lee D, Graham BS, Chiu YL, et al. Breakthrough infections during phase 1 and 2 prime-boost HIV-1 vaccine trials with canarypox vectors (ALVAC) and booster dose of recombinant gp120 or gp160. J Infect Dis 2004;190:903-7.
31. Van Rompay KK, Abel K, Lawson JR, et al. Attenuated poxvirus-based simian immunodeficiency virus (SIV) vaccines given in infancy partially protect infant and juvenile macaques against repeated oral challenge with virulent SIV. J Acquir Immune Defic Syndr 2005;38:124-34.
32. Pal R, Venzon D, Letvin NL, et al. ALVAC-SIV-gag-pol-env-based vaccination and macaque major histocompatibility complex class I (A*01) delay simian immunodeficiency virus SIVmac-induced immunodeficiency. J Virol 2002;76:292-302.
33. Pal R, Venzon D, Santra S, et al. Systemic immunization with an ALVAC-HIV-1protein boost vaccine strategy protects rhesus macaques from CD4+ T-cell loss and reduces both systemic and mucosal simian-human immunodeficiency virus SHIVKU2 RNA levels. J Virol 2006;80:3732-42.
34. Gilbert PB, Ackers ML, Berman PW, et al. HIV-1 virologic and immunologic prognostic assessment of antiretroviral therapy among HIV-1-infected subjects in a trial of the efficacy of recombinant glycoprotein 120 vaccine. J Infect Dis 2005;192:974-83.
35. Gilbert PB, Peterson ML, Follmann D, et al. Correlation between immunologic responses to a recombinant glycoprotein 120 vaccine and incidence of HIV-1 infection in a phase 3 HIV-1 preventive vaccine trial. J Infect Dis 2005;191:666-77.
36. Flynn NM, Forthal DN, Harro CD, Judson FN, Mayer KH, Para MF. Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. J Infect Dis 2005;191:654-65.
37. Buchbinder SP, Mehrotra DV, Duerr A, et al. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. Lancet 2008;372:1881-93.
38. Mascola JR, D'Souza P, Gilbert P, et al. Recommendations for the design and use of standard virus panels to assess neutralizing antibody responses elicited by can-

didate human immunodeficiency virus type 1 vaccines. J Virol 2005;79:10103-7.
39. Forthal DN, Gilbert PB, Landucci G, Phan T. Recombinant gp120 vaccine-induced antibodies inhibit clinical strains of HIV-1 in the presence of Fc receptor-bearing effector cells and correlate inversely with HIV infection rate. J Immunol 2007;178:6596-603.
40. McElrath MJ, De Rosa SC, Moodie Z, et al. HIV-1 vaccine-induced immunity in the test-of-concept Step Study: a case-cohort analysis. Lancet 2008;372:1894-905.
41. Seder RA, Darrah PA, Roederer M. T-cell quality in memory and protection: implications for vaccine design. Nat Rev Immunol 2008;8:247-58. [Erratum, Nat Rev Immunol 2008;8:486.]
42. Salazar-Gonzalez JF, Salazar MG, Keele BF, et al. Genetic identity, biological phenotype, and evolutionary pathways of transmitted/founder viruses in acute and early HIV-1 infection. J Exp Med 2009; 206:1273-89.
43. Karnasuta C, Paris RM, Cox JH, et al. Antibody-dependent cell-mediated cytotoxic responses in participants enrolled in a phase I/II ALVAC-HIV/AIDSVAX B/E prime-boost HIV-1 vaccine trial in Thailand. Vaccine 2005;23:2522-9.
44. Ratto-Kim S, Loomis-Price LD, Aronson N, et al. Comparison between env-specific T-cell epitopic responses in HIV-1-uninfected adults immunized with combination of ALVAC-HIV(vCP205) plus or minus rgp160MN/LAI-2 and HIV-1-infected adults. J Acquir Immune Defic Syndr 2003;32:9-17.
45. Alter G, Teigen N, Ahern R, et al. Evolution of innate and adaptive effector cell functions during acute HIV-1 infection. J Infect Dis 2007;195:1452-60.
46. Goonetilleke N, Liu MK, Salazar-Gonzalez JF, et al. The first T cell response to transmitted/founder virus contributes to the control of acute viremia in HIV-1 infection. J Exp Med 2009;206: 1253-72.